Proof of Concept to Isolate and Culture Primary Muscle Cells from Northern Elephant Seals to Study the Mechanisms that Maintain Aerobic Metabolism Under the Hypoxic Conditions of Breath-hold Diving

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LONG-TERM GOALS

To isolate and culture primary muscle cells from the swimming muscles of northern elephant seals.

OBJECTIVES

Objective 1. To test the hypothesis that the skeletal muscle cells of diving mammals are pre-conditioned to function under hypoxic conditions compared to terrestrial models. Specifically, proteins and transcripts associated with increased oxygen storage, lipid catabolism and hypoxic pathways will be upregulated in seal primary cells as compared to terrestrial models.

Objective 2. To test the hypothesis, that varying levels of stimulation and/or hypoxic conditions will differentially regulate the expression of myoglobin.

APPROACH

This work will be carried out in collaboration with Drs Daniel Costa, and Daniel Crocker. We will collect muscle biopsies from animals that are being sedated for their projects. By utilizing the same animals and working with these collaborators we will minize the impact and number of animals being handled at Año Nuevo.

Muscle sampling
Biopsy samples of approximately 50 mg will be collected with a 6-mm biopsy cannula (Depuy, Warsaw, Indiana) from the swimming (M. longissimus dorsi) muscle. Before the biopsy is taken, the skin is first cleaned with betadine scrub. A small area the size of a dime is shaved and one ml of 2% Lidocaine; to minimize the trauma to the biopsy site, is then injected beneath the skin around the biopsy site. The skin is punctured with a #10 scalpel and the biopsy needle inserted to a depth of 3-4 inches (the blubber layer is typically 2 inches thick). Once collected, the biopsy will be dipped in 100% ethanol for sterilization then placed into culture media (F10 or F10 plus lipids) and stored on ice.

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After collection the biopsy site will be cleaned with betadine scrub. Slight pressure is applied to the biopsy site after flushing with 4x4 gauze pads to stop any minor bleeding.

The biopsy samples will then be shipped overnight on ice to the lab at Colorado State University for primary cell isolation and culturing.

Primary Personnel: Shane B. Kanatous, Amber Schlater, Teresa Garcia and Caitlin Kielhorn

WORK COMPLETED

We successfully sample 10 animals over a two day period in September, 2012. The first major huddle to the study was to determine the effect of the overnight shipping of the viability of the samples and the ability to isolate cells from the biopsies. That huddle was met with tremendous success both media preps; the F10 and F10 +lipids, produced cells from both the tissue samples and the transfer media (see representative picture below).

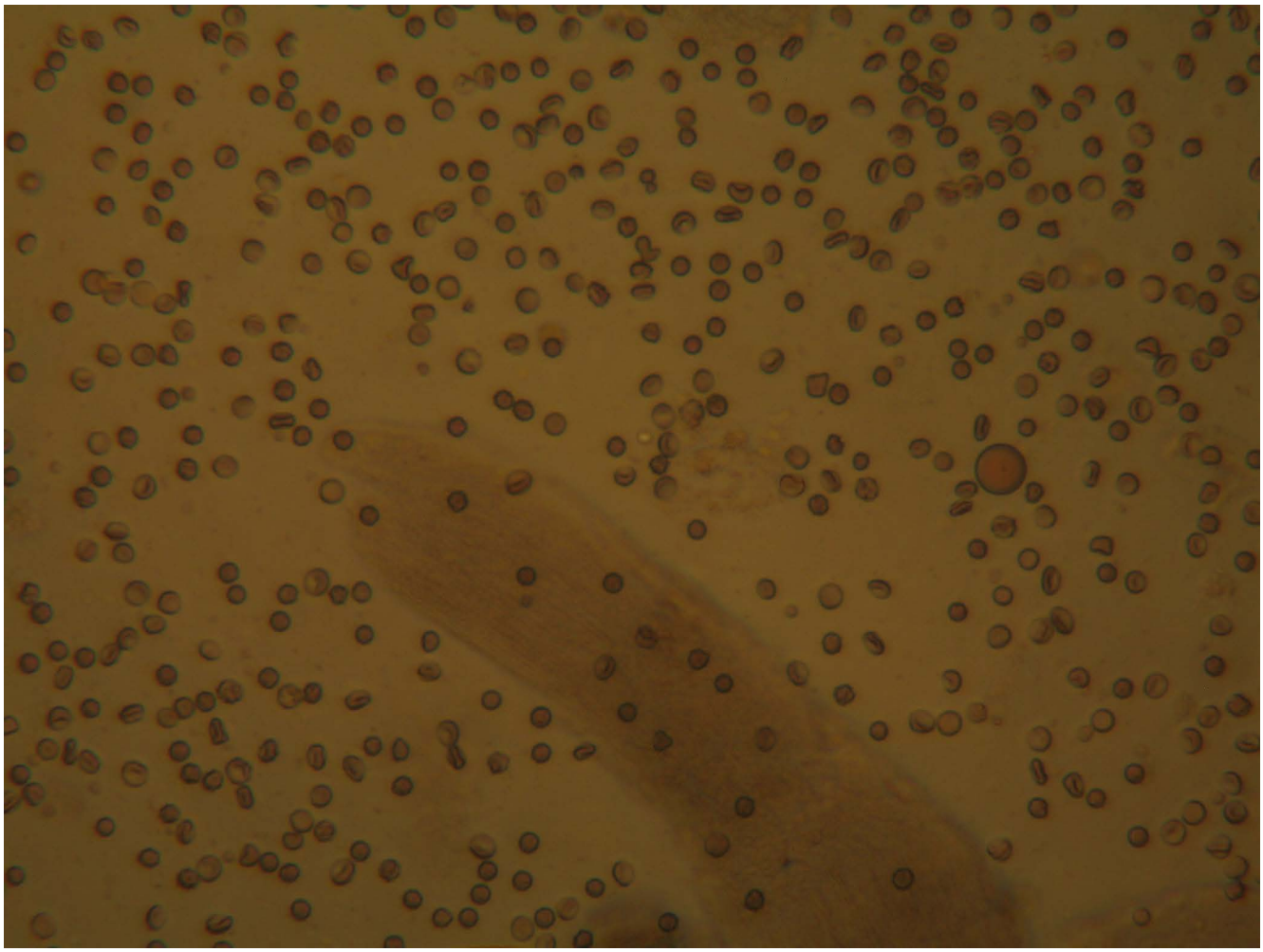

Figure 1: Representative image of cells isolated from a skeletal muscle biopsy of a northern elephant seal. The first major huddle of the project was to determine if we would be able to obtain viable cells from the biopsies after being shipped overnight back to Colorado State University from the field site at Ano Nuevo, California. In the image numerous different cell types are seen floating above a piece of the muscle biopsy.

Unfortunately after that we had some contamination issues during the proliferation stage. I think those issues could be easily resolved with the addition of not only antibiotics but an antifungal to the transfer and culturing medias. So bottom line, with just a few adjustments to the culturing media and conditions, I firmly believe we will be successful in the future. We currently plan to sample again in the late January or February 2013 timeframe.

RESULTS

N/A

IMPACT/APPLICATIONS

Significance: Understanding the regulation of oxygen metabolism and its effects on development and animal performance is of vital importance to understanding the physiological plasticity of organisms in a changing environment. The results of this study will decipher whether the regulation of the expression of myoglobin is determined more by evolutionary history or environmental stimuli.

Implications and application beyond our current research: The techniques being developed here will have broad applications to other non-model organisms which cannot easily be exposed to different experimental conditions. The use of primary cells can be expanded to other cell types that could be obtained safely from animals using biopsy techniques. In addition, an unexplored area that could evolve from this technique is to expose the cultured cells to other environmental stresses such as the different aspects of climate change (i.e. temperature, salinity, pH, oxygen and carbon dioxide tensions), changes in substrate utilization or exposure to different contaminants to determine their effect on growth and development.